REMARKS

Claims 1 – 45 are pending. Reconsideration and allowance of the present application based on the following remarks is respectfully requested.

Applicants are pleased to note that the Examiner indicated that claims 28, 30-32 and 34-39 would be allowable if rewritten in independent form.

Applicants note that claims 2-8 which depend directly or indirectly from claim 1, claims 10-14 which depend directly or indirectly from claim 9, claims 17-22 which depend directly or indirectly from claim 16 were only rejected under § 112, second paragraph. Therefore, it is Applicants' understanding that claims 2-8, 10-14 and 17-22 would be allowable if the § 112, second paragraph is overcome.

Title:

Applicants have amended the title to read as follows "GENE EXPRESSION DATA ANALYSIS USING FUZZY LOGIC." Therefore, Applicants respectfully submit that the present title is indicative of the invention to which the claims are directed, and request that the objection to the title be withdrawn.

Specification:

The specification was objected to because it contained an embedded hyperlink and/or browser executable code. Accordingly, Applicants have amended the paragraph of the specification containing the hyperlink or browser executable code and removed the hyperlink feature from the paragraph.

Claim indefiniteness:

Claims 1-22 and 42-44 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants have amended claim 1 to clarify the claim language further. Applicants have replaced the phrase "higher number" by the word "plurality." Claim 1 recites, *inter-alia*, "receiving sets of expression data derived from control and treatment sets of cell-derived samples as crisp input data, said sets of expression data representing a direction and a magnitude of regulation of each one of a plurality of different genes or proteins." Claim 16 recites, *inter-alia*, "receive sets of expression data derived from control and treatment sets of cell-derived

samples as crisp input data, said sets of expression data representing a direction and a magnitude of regulation of each one of a plurality of different genes or proteins."

The Examiner contends that the expression data is described as being "cell-based" which at best only cites a single cell. The Examiner assumes that multiple cells are meant. Applicants submit that the Examiner's assumption is correct. Claims 1 and 9 recite "cell-derived samples." Applicants respectfully submit that one of ordinary skill in the art while reading the claims would understand that cell-derived samples refers to one or more cells. The phrase "cell-derived" is an adjective meaning that the samples are derived from multiple cells. For example, in the parallel phrase "cell-mediated immune response which refers to the immune response produced when sensitized T cells directly attack foreign antigens and secrete lymphokines,"[1] (emphasis added), the adjective "cell-mediated" refers to multiple cells. See also, a copy of a scientific journal article attached hereto as Exhibit A.

Therefore, Applicants respectfully submit that claims 1-22 and 42-44 are in full compliance with § 112 and respectfully request that the rejection of claims 1-22 and 42-44 under § 112, second paragraph be withdrawn.

35 USC 103 Rejection

Claims 1, 9, 16, 23-27, 29, 33 and 40-45 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Ramm et al. (U.S. Patent No. 6,345,115) in view of Agrafiotis et al. (U.S. Patent No. 6,421,612). Applicants respectfully traverse this rejection for at least the following reasons.

Ramm et al. merely mentions the usage of a fuzzy logic algorithm. However, as acknowledged in the Office Action, Ramm et al. lacks specifics of what is practiced in such a fuzzy logic algorithm.

The Office Action contends that Agrafiotis et al. discloses the specifics of a fuzzy logic algorithm including fuzzifying a crisp input data to provide fuzzified values, applying a set of heuristic rules to the fuzzified values to generate a predicted value of a data point C, deffuzzifying the predicted value of C and determining a confidence level of the predicted value of C. Applicants respectfully disagree.

---

[1]Excerpted from *The American Heritage Dictionary of the English Language, Third Edition* Copyright © 1992 by Houghton Mifflin Company. Electronic version licensed from Lernout & Hauspie Speech Products N.V.

Agrafiotis et al. discloses a fuzzy logic algorithm in which input variables (encoded structure data 826, which is crisp) are first converted into fuzzy sets by a fuzzification unit 1008 using fuzzy set definitions in data base 1006. Then a fuzzy inference module 1010 evaluates all the rules (IF-THEN rules with one or more antecedent and consequent variables, see col. 20, lines 54-55 in Agrafiotis et al.) in a rule base 1004 to produce an output by performing the following steps: determining the degree of match between the fuzzified input data and the fuzzy sets defined for the input variables in data base 1006, calculating the firing strength of each rule based on the degree of match of the fuzzy sets computed and the connectives (AND, OR etc.) used in the fuzzy rule, and deriving the output based on the firing strength of each rules computed and the fuzzy sets defined for the output variable in the data base 1006. The fuzzy output of the fuzzy inference module 1010 is then defuzzified by defuzzification unit 1012 using the output fuzzy set definitions in data base 1006 and a defuzzification strategy. (see col. 21, lines 55-67 and col. 22, lines 1-13 in Agrafiotis et al.).

Agrafiotis et al. does not disclose, teach or suggest applying a set of heuristic rules to the fuzzified values to generate a predicted value of a data point, defuzzyfying the predicted value of the data point and determining a confidence level of the predicted value of the data point. Agrafiotis et al. clearly does not determine a confidence level of the predicted value of a data point.

Consequently, Agrafiotis et al. does not disclose, teach or suggest, *inter-alia*, "applying a set of heuristic rules to the fuzzified values to generate a predicted value of a data point C; defuzzifying the predicted value of C; and determining a confidence level of the predicted value of C," as recited in claim 1.

Agrafiotis et al. does not disclose, teach or suggest, *inter-alia*, "a heuristic rules applier for applying a set of heuristic rules to the fuzzified values to generate a predicted value of a data point C; a defuzzifier for defuzzifying the predicted value of C; and a confidence level determiner for determining a confidence level of the predicted value of C," as recited in claim 9.

Agrafiotis et al. does not disclose, teach or suggest, *inter-alia*, "apply a set of heuristic rules to the fuzzified values to generate a predicted value of a data point C; defuzzify the predicted value of C; and determine a confidence level of the predicted value of C," as recited in claim 16.

Agrafiotis et al. does not disclose, teach or suggest, *inter-alia*, "applying a set of heuristic rules to the fuzzy data values to generate a predicted data value having a defined relationship to

the two or more fuzzy data values; defuzzifying the predicted data value to generate a crisp predicted data value; and searching the differential gene expression data for a data value that substantially matches the crisp predicted data value," as recited in claim 23.

Agrafiotis et al. does not disclose, teach or suggest, *inter-alia*, based on the plurality of quantitative values, obtaining a plurality of qualitative descriptors; based on application of a predetermined model to the plurality of qualitative descriptors, obtaining at least one predicted value, each said predicted value relating to at least one other sequence of at least one of the group consisting of nucleic acids and amino acids; and determining a confidence level of each said predicted value," as recited in claim 24.

Therefore, neither Ramm et al. nor Agrafiotis et al., alone or in combination disclose, teach or even suggest the suggest the subject matter recited in claims 1, 9, 16, 23 and 24.

Therefore, Applicants respectfully submit that claims 1, 9, 16, 23 and 24 and claims 25-27, 29, 33 and 40-45 which depend directly or indirectly from one of claims 1, 9, 16 and 24 are patentable. Thus, Applicants respectfully request that the rejection of claims 1, 9, 16, 23-27, 29, 33 and 40-45 under § 103(a) over the combination of Ramm et al. and Agrafiotis et al. be withdrawn.

CONCLUSION

All rejections have been addressed. It is respectfully submitted that the present application is in condition for allowance, and a notice to that effect is earnestly solicited.

Should there be any questions or concerns regarding this application, the Examiner is invited to contact the undersigned at the below-listed telephone number.

Please charge any fees associated with the submission of this paper to Deposit Account Number 03-3975 under Order No. 070441/0274072. The Commissioner for Patents is also authorized to credit any over payments to the above-referenced Deposit Account.

Respectfully submitted,

PILLSBURY WINTHROP LLP

By: _____

Robert C. Perez
Reg. No.: 39,328
Tel. No.: (703) 905-2159
Fax No.: (703) 905-2500

RCP/KG
1600 Tysons Boulevard
McLean, VA 22102
(703) 905-2000

Customer Number [00909]

EXHIBIT A

EXHIBIT-A

Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei

David Humpherys*†, Kevin Eggan*†, Hidenori Akutsu‡, Adam Friedman*, Konrad Hochedlinger*, Ryuzo Yanagimachi‡, Eric S. Lander*†, Todd R. Golub*§¶, and Rudolf Jaenisch*†‖

*Whitehead Institute for Biomedical Research and †Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, MA 02142; ‡Department of Anatomy and Reproductive Biology, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96822; §Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA 02115; and ¶Department of Pediatrics, Harvard Medical School, Boston, MA 02115

Contributed by Eric S. Lander, July 22, 2002

To assess the extent of abnormal gene expression in clones, we assessed global gene expression by microarray analysis on RNA from the placentas and livers of neonatal cloned mice derived by nuclear transfer (NT) from both cultured embryonic stem cells and freshly isolated cumulus cells. Direct comparison of gene expression profiles of more than 10,000 genes showed that for both donor cell types ≈4% of the expressed genes in the NT placentas differed dramatically in expression levels from those in controls and that the majority of abnormally expressed genes were common to both types of clones. Importantly, however, the expression of a smaller set of genes differed between the embryonic stem cell- and cumulus cell-derived clones. The livers of the cloned mice also showed abnormal gene expression, although to a lesser extent, and with a different set of affected genes, than seen in the placentas. Our results demonstrate frequent abnormal gene expression in clones, in which most expression abnormalities appear common to the NT procedure whereas others appear to reflect the particular donor nucleus.

The majority of cloned mammals derived by nuclear transfer (NT) die during gestation, display neonatal phenotypes resembling large offspring syndrome (1, 2), often with respiratory and metabolic abnormalities, and have enlarged and dysfunctional placentas (3–5). For a donor nucleus to support development in a clone, it must be reprogrammed to a state compatible with embryonic development. The transferred nucleus must properly activate genes important for early embryonic development and also adequately suppress differentiation-associated genes that had been transcribed in the original donor cell. Because few clones survive to birth, the question remains whether survivors are normal or merely the least severely affected animals, making it to adulthood despite harboring subtle abnormalities originating from inadequate nuclear reprogramming (6).

Given the long generational time of most animal species cloned, the long-term consequences of cloning on health have been difficult to assess. Evidence that cloned animals retain abnormalities capable of causing severe health consequences has been obtained for mice cloned from Sertoli cells that, in comparison to normally developing controls of the same sex and background, had reduced lifespans and frequent pneumonia and hepatic failure (7). Additionally, mice cloned from cumulus cell donor nuclei were obese with increased body fat and size (8). Because obesity was not passed on to the offspring of the clones it is unlikely to reflect any genetic changes in the clones but instead to reflect epigenetic abnormalities arising from inadequate nuclear reprogramming. Examination of adult clones in other species has been described only for younger animals and limited to physical examinations and blood and urine chemistry (9).

Development of clones derived from embryonic stem (ES) cell nuclei to the blastocyst stage is less efficient than that of clones derived from somatic donor nuclei because the majority of ES cells are in S phase (6), a stage of the cell cycle that is incompatible with survival of clones (10). However, survival to birth or adulthood of blastocysts derived from ES cell donor nuclei is about 10–20 times more efficient than that of clones derived from somatic donor nuclei (11, 12). This striking increase in development rate suggests that less reprogramming is needed for nuclei of embryonically derived cells and that reprogramming is important for postimplantation development. Despite this enhanced developmental rate, it has been argued that epigenetic instability described in ES cells during *in vitro* culturing (13, 14) makes them a poor choice for NT donors (15). However, this argument is based largely on the expression of imprinted genes known to be particularly affected in ES cells. Nevertheless, common phenotypes, including dramatically overgrown placentas, have been described when using either ES cell or somatic cell donor nuclei for NT (3, 12).

Examination of gene expression in cloned animals has largely been limited to preimplantation embryos for a small number of genes important for early embryogenesis (16–18). In clones surviving to birth, the expression of a limited number of imprinted genes has been described, and several are expressed at abnormal levels (14, 15) with some changes reflecting epigenetic, in addition to chromosomal, abnormalities (19) arising in donor cells, in particular during the *in vitro* culture of ES cell donors. However, apart from about a dozen examined genes, it is not clear to what extent other imprinted gene expression or global gene expression may be abnormal in neonatal clones. Faulty imprinting has been proposed as a candidate for some cloning phenotypes because imprinted genes are frequently involved in fetal and placental growth (20) and are likely resistant to reprogramming because their imprints are established in the germ line and specifically maintained in the embryo (21). Furthermore, *in vitro* culturing of embryos can lead to a loss of imprinting and large offspring syndrome (22, 23). Because cloned embryos also display phenotypes resembling large offspring syndrome it is possible that some of these phenotypes result from imprinting abnormalities.

We report here the expression profiles of more than 10,000 genes in placentas and livers of neonatal clones from both ES cell and cumulus cell donor nuclei. Our results suggest that many expression abnormalities are common to the NT procedure whereas some reflect the particular donor nucleus. These results further emphasize the severity of placental dysfunction and illustrate abnormalities in clones surviving to birth.

Materials and Methods

RNA Preparation and Array Hybridization. Cloned mouse neonates were produced by NT from ES and cumulus cell nuclei. Most clones derived from both donor cell types exhibited fetal overgrowth and an enlarged placenta. The average birth and placental weights, respectively, were 1.3 g and 0.09 g for normally fertilized embryos, 2.1 g and 0.32 g for ES cell NT mice (12), and 2.2 g (n = 12) and 0.33 g (n = 14) for cumulus cell NT mice. The distribution of increased placental and birth weights was similar to that seen in our previous study (14). RNA was isolated from a total of 24 placentas and 20 livers, and expression analysis was performed by using Affymetrix (Santa Clara, CA) gene arrays. Two sets of experiments

Abbreviations: NT, nuclear transfer; ES, embryonic stem.

‖To whom reprint requests should be addressed. E-mail: jaenisch@wi.mit.edu.